AR201-13437B

Robust Summary - Group 6: Low Benzene Naphthas

Acute Toxicity

Test Substance	Heavy Aromatic Distillate, CAS# 64742-48-9
Method	
Method/guideline followed	No guideline specified, comparable to standard study
Type (test type)	Acute LD50
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex per dose	5/dose/group (4 groups)
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Rats were dosed once with undiluted heavy aromatic distillate at 4.5, 5.0, 5.5 and 6.0 g/kg, and were observed for 14 days post dosing for mortality, moribundity and clinical signs. Body weight was obtained at initiation, and after 7 and 14 days post dosing. Gross necropsies were performed on all rats at study termination.
Results	
LD_{50} with confidence limits.	There were no deaths attributed to test article administration, and therefore, the LD50 was not reached in either sex at the highest dose. Body weight was not significantly changed over the 14-day observation period. Over the first week of study, there were instances of perianal soiling, dry material around the mouth, and soft feces. In sacrificed rats, there were no findings that could be attributable to test material administration.
Remarks	
Conclusions	
(study author)	The LD50 was not reached at the highest dose of 6.0 g/kg.
Data Quality	
Reliability	1. Reliable without restrictions
References	Rausina, G. 1984. Acute oral toxicity study in albino rats using heavy aromatic distillate. Proj. # 2049. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX.
Other	
Last changed	Rev 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary – Group 6: Low Benzene Naphthas

Acute Toxicity

Test Substance	Heavy aromatic distillate, CAS# 64742-48-9. No composition or purity analysis reported, refer to sponsor.
Method	
Method/guideline followed	No guideline specified but comparable to standard study
Type (test type)	Acute LC_{50}
GLP	Yes
Year	1983
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex/dose	5
Vehicle	filtered air
Route of administration	whole body inhalation
Test Conditions	Six groups of 10 rats (5M, 5F/group, 12-16 wks old, 145-307g) were individually housed and exposed in stainless steel/glass inhalation chambers to aerosolized test article or filtered air for 4 hours, followed by 14 days of observation post exposure for clinical signs, morbidity and death. Non-fasted rats were sacrificed on day 14 and necropsied for gross lesions. Nominal chamber concentrations (g/m^3) were 0.0, 12.2, 24.8, 25.8, 19.6, and 99 (uncorrected for large particle condensation), but actual chamber concentrations were 0, 6.0, 7.6, 8.6, 9.1, and 11.2 as determined by gas chromatography. Probit analysis was used to estimate an LC_{50}.
Results	
LC_{50} with confidence limits.	LC_{50} was estimated to be 8.5 g/m^3 (actual chamber concentration).
Remarks	There was a large difference between nominal and actual concentrations in the inhalation chambers that was not addressed in the report. In the analyses, exposure concentration was estimated by comparing peak height (rather than peak area) with that of the neat sample. The method of calculating chamber concentration of test article in ppm was not reported (Comment by contractor). All animals in the high dose group died during exposure with congestion of lungs and nasal turbinates with red discharge. Six animals died in groups 4 and 5 during exposure, and were found with gas in the G.I. tract. Mean body wt of males and females decreased by day 7 but then increased over the remaining 7 days. Most rats exhibited nasal and ocular discharges, and in the higher dose groups showed signs of CNS effects, (hyperexcitablility, twitching, circling) that . were absent by day 2. Other clinical effects were absent by day 7. No test article related gross pathological lesions were observed.
Conclusions	LC_{50} was 8.5 g/m^3.
Data Quality	
Reliability	**1**. Reliable without restrictions. This study is acceptable for range-finding since the concentrations employed yielded a dose response curve covering the full range of biological response (0-100% fatalities).
References	Goode, J.W. 1983. LC_{50} Inhalation toxicity study in rats using heavy aromatic distillate. Proj. # 2050. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	Rev. 7/2/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 6: Low Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Heavy Aromatic Distillate, Gulf. CAS #64742-48-9. Water-white liquid with characteristic aromatic odor. Composition analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1984
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD
Quantity	1.0mg S9 fraction/ml treatment medium (0.3ml S9 fraction in 3 ml medium/flask)
Induced or not induced	Aroclor 1254 induced (treatment not specified)
Concentrations tested	Cytotoxicity, final conc. (trial 2): 128, 256, 512, 1024µg/ml ± S9; Mutagenicity, final conc. (trial 2): 64, 128, 256, 512, 750, 1024µg/ml –S9; 128, 256, 512, 1024, 1500, 2048µg/ml +S9, all diluted in 10% Pluronic® polyol F68 (prepared in dionized water, mol. wt. 8350)
Statistical Methods	Frequency of mutant colonies per million clonable cells was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979). Criteria for positive results were significant ($p<0.05$) increase in mutant colonies (HGPRT+/-? HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
Remarks for Test Conditions	Sufficient Heavy Aromatic Distillate (HAD) was weighed separately for each dose level into 10 ml volumetric flasks, 1.8ml of 10% F68 added per ml of final volume and medium (Ham's F-12 without hypoxanthine) added as required to achieve final 10ml volume for testing. All dosing preparations were vortexed just after addition of medium and just prior to use when 20µl of each preparation was added to 3ml treatment medium/culture vessel. All cultures were incubated at 37^0C in 5% CO_2 enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For cytotoxicity, each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with $5x10^5$ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10^6 cells were exposed to HAD for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10^5-10^6 cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and $2x10^5$ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10^{-5}M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of

	mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.
Results Genotoxic effects	First trial was aborted after post treatment cell counts in the mutagenicity assay, because of disparity in cytotoxic response between cytotoxicity aspect and mutagenicity aspect of the trial. No cytotoxicity was seen in the mutagenicity aspect. Results of the second cytotoxicity trial demonstrated cytotoxicity at 512 µg/ml and higher in the –S9 portion and only a slight decrease in cell count at 1024 µg/ml in the activated portion but no cytotoxicity in colony count after treatment. The second mutagenicity trial indicated post treatment cell death in –S9 cultures beginning at 64 µg/ml with significant effects at 256 µg/ml and 100% toxicity at 750 µg/ml. In +S9 cultures, slight cell toxicity was seen at 1500 µg/ml and above. For both activated and non-activated cultures, no significant dose-related responses or significant increase in mutant frequency were observed at any dose level of HAD. Positive control compounds responded appropriately (EMS 5.2 fold increase and B(a)P 2.8 fold increase over vehicle control).
Conclusions (contractor)	Mutagenicity was not observed at any HAD dose level tested with or without metabolic activation. Heavy Aromatic Distillate does not induce gene point mutations in the CHO/HGPRT test under conditions of this assay.
Data Quality *Reliabilities*	**1**. Reliable without restrictions. Study conforms to standard design. GLPs have been followed
Reference	Papciak, M.S., Goode, J.W. 1984. CHO/HGPRT test: Heavy Aromatic Distillate. Proj. #2054. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275.
Other *Last changed*	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 6: Low Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Heavy Aromatic Distillate, Gulf. CAS #64742-48-9. Water-white liquid with characteristic aromatic odor. Composition analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1984
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml; Transformation: 16, 32, 64, 200µg/ml, all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt. 8350, 80% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
Remarks for Test Conditions	Sufficient Heavy Aromatic Distillate (HAD) was weighed separately for each dose level, 0.45ml of 10% F68 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For cytotoxicity, 2 plate cultures/dose group, 2 plate cultures for vehicle F68 or medium negative control were seeded with $1x10^4$ cells/plate in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flasks ($1x10^4$ cells/flask/dose group)) and two cloning flasks (100 cells per flask/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation flask cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Cloning flask cultures were fixed, stained, and counted visually on day 8 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Flask cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.
Results	
Genotoxic effects	In the first trial, HAD induced toxicity in BALB/3T3 cells after two days exposure beginning at 32µg/ml (59.9% relative survival), increasing with dose level to 2.9% relative survival at 5000µg/ml. The first trial was discarded due to loss of many cultures (27/105) due to contamination. Results of the second transformation trial indicated no treatment related cell transformation induced by HAD. Toxicity was evident 32µg/ml (67.2% relative cloning efficiency), increasing sharply at 200µg/ml (28.8% cloning efficiency). Positive and negative controls gave expected results.
Conclusions (contractor)	Heavy Aromatic Distillate did not induce transformation in BALB/3T3 cells at any dose level under conditions of this assay.
Data Quality	
Reliabilities	**1.** Reliable without restriction. Study conforms to standard design. GLPs have been followed.

Reference	Brecher, S., Good, J.W. 1984. BALB/3T3 transformation test: Heavy Aromatic Distillate. Proj. #2057. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenes is 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.
Other	
Last changed	4/11/2001 (prepared by a contractor to the Olefins Panel).

Robust Summary - Group 6: Low Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Heavy Aromatic Distillate, Gulf. CAS #64742-48-9. Water white liquid with characteristic aromatic odor. Composition analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Standard method based on Williams et al. (1977,1982)
Type	In vitro mammalian cell DNA repair assay
System of testing	Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (13-14 wks old) – 1 rat per test
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Range-finding: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000 µg/ml: UDS assay 10, 40, 100, 200 µg/ml; all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt 8350, 80% hydrophilic)
Exposure period	18 hours
Statistical Methods	None employed. Criteria for positive response are incorporation of radioactive precursor (^{3}H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. A positive response need not be dose related.
Remarks for Test Conditions	Sufficient Heavy Aromatic Distillate (HAD) was weighed separately for each dose level, 0.45ml of 10% F68 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30µl to each 3 ml culture. The conc. of ^{3}H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37^{0}C in 5% CO_2 enriched humidified atmosphere. For range-finding, primary hepatocytes derived from freshly perfused rat liver were seeded (approx. 1x10^5 cells/ml) into treatment vessels, exposed to test material for 18 hours (2 cultures/dose level; 2 untreated cultures, and two vehicle (F68) control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1x10^5 cells/ml were seeded into coverslip cultures, exposed to ^{3}H-thymidine and test substance for 18 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (0.2µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8^{0}C. Autoradiographs were developed, stained and coverslipped on day 14. Numbers of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted and this number was divided by a conversion factor of 2, to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷3) were calculated.
Results	
Genotoxic effects	HAD induced toxicity in primary hepatocytes beginning at 32-64µg/ml (72-80% relative viability) after 18 hours exposure, which increased with dose levels to 2% viability at 5000µg/ml. HAD did not cause unscheduled DNA synthesis at any dose level. Positive and negative controls gave expected results.
Conclusions	
(contractor)	Unscheduled DNA synthesis was not observed in primary culture of rat hepatocytes at any dose level of Heavy Aromatic Distillate, indicating that this material does not damage DNA under conditions of this assay.

Data Quality *Reliabilities*	**1**. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.
Reference	Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of heavy aromatic distillate. Proj. #2056. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Williams, G.M. 1977. Cancer Res. 37: 1845-1851 Williams et al. 1977. In Vitro 13: 809-817 Williams et al. 1982. Mut. Res. 97:359-370
Other *Last changed*	4/11/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 6: Low Benzene Naphthas

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Heavy Aromatic Distillate, Gulf CAS #64742-48-9. Water white liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor.
Method	
Method/guideline followed	Comparable to standard assay
Type	Mammalian bone marrow erythrocyte micronucleus
GLP	Yes
Year	1984
Species	Mouse
Strain/Sex	Crl:CD®–1 (ICR) BR Swiss: Male and female: Range finding (RF): 2M, 2F/group; Micronucleus:.10M, 10F/group; 15M, 15 F in 1 group
Route of administration	Oral gavage
Doses/concentration levels	RF: 0, 1.25, 2.5, 5.0 g/kg in corn oil: Micronucleus: 0, 0.625, 1.25, 2.5 g/kg in corn oil
Exposure period	1 dose/day for 2 days; 1 group at 2.5 g/kg 1 dose, 1 day only
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	Heavy Aromatic Distillate (HAD) dosing solutions were prepared fresh for each day of dosing –12.5 g HAD (RF) or 6.25 g HAD (micronucleus) mixed with corn oil to make 50 ml, blended by shaking. Based on results of the range finding study, three groups of mice were given HAD by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 2.5 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald/ Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	
Genotoxic effects	In range finding test, 1/2males and ½ females died at 5.0 g/kg dose level by day 3. In the micronucleus test, 1/10 females in the 2.5 g/kg dose group (2 days of dosing) died by day 4. All other mice survived to study sacrifice. Body wts were comparable to negative controls for both sexes in all treatment groups and positive controls. Treatment with HAD did not show any significant changes in micronucleus formation or in the ratio of PCE/NORM at any dose level. Average PCE/NORM ratio was 0.9% for all HAD treatment groups and negative control; ratio for positive control was 0.5%.
NOAEL (NOEL)	NOEL (systemic) = 1.25 g/kg: NOEL (genetic) = 2.5 g/kg
LOAEL (LOEL)	
Conclusions	
(study authors)	Oral treatment of mice with Heavy AaromaticDistillate for 1 or 2 days at doses up to 2.5 g/kg did not cause increased frequency of micronucleated polychromatic erythrocytes in bone marrow of treated mice. Under these test conditions, Heavy Aromatic Distillate does not induce cytogenetic damage.
Data Quality	
Reliabilities	**1.** Reliable without restrictions. Study conforms to standard design. GLP followed.
References	Khan, S.H. and Goode, J.W. 1984. Micronucleus test in mouse bone marrow: Heavy

Other *Last changed*	Aromatic Distillate administered orally for 2 days. Proj. #2005. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Rev. 6/25/2001 (Prepared by a consultant to the Olefins Panel)

Robust Summary - Group 6: Low Benzene Naphthas

Repeated Dose Toxicity

Test Substance	Heavy Aromatic Distillate, CAS # 64742-48-9. No analysis of purity or composition
Remarks	reported; referred to sponsor.
Method	
Method/guideline followed	No guidelines specified, comparable to standard study
Test type	Subacute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Whole body inhalation
Duration of test	5 days
Doses/concentration levels	0, 1.2, 2.7, 5.0 g/m^3
Sex	Males and females 5/sex/group
Exposure period	5 days
Frequency of treatment	6 hours/day
Control group and treatment	filtered air at 6 hrs/day for 5 days
Post exposure observation period	None
Statistical methods	Analysis of Variance, Dunnett's test
Test Conditions	Animals (13 weeks old at study initiation, 156-279g) were housed individually in screen-bottom cages with automatic watering in rooms maintained at approx. 74^0F with relative humidity of 50%, and 12 hour light/ dark cycle. Chow diet and water were provided ad lib except during exposure. Chamber concentrations were monitored by GC; peak areas were compared with those of neat test article standards. Rats were monitored twice daily for morbidity and mortality, and observed once daily for clinical signs. Body weights were measured at initiation and termination. Necropsies were performed for gross lesions.
Results	
NOAEL (NOEL)	NOEL not determined
LOAEL (LOEL)	LOEL = 1.2 g/m^3 based on clinical observations: perianal staining, red material around
Remarks	nose/mouth, ocular porphyrin. (assessed by Reviewer). One female rat in the high dose group died during the initial exposure; all other rats survived until termination. Males and females exposed to 5.0g/m3, showed a dose related weight loss of approx. 8% after 5 days of dosing. Incidence of dry red material around nose/mouth, ocular porphyrin, clear discharge from the eyes, partially closed eyes and perianal staining occurred in all groups receiving test article. The two high dose groups showed purulent discharge from the eyes and bloody tears. Reviewer comment: The total incidence of clinical observations increased in a manner related to exposure concentration.
Conclusions	One death occurred during exposure of the high dose group. All other animals exhibited clinical signs that included ocular discharge, eye closure, and dry red material around the nose/mouth. Gross pathological lesions were not observed which could be directly attributable to test article administration.
Quality	
Reliabilities	1. Reliable without restrictions.
References	Gordon, T. 1983. One week repeat dose inhalation toxicity study in the rat using heavy aromatic distillate. Proj. # 2062. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX.
Other	
Last changed	Rev. 7/3//2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 6: Low Benzene Naphthas

Repeated Dose Toxicity

Test Substance	Heavy Aromatic Distillate, CAS #64742-48-9. No composition or purity analysis reported;
Remarks	refer to sponsor.
Method	
Method/guideline followed	No guideline specified; comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1985
Species	Rat
Strain	Fischer 344
Route of administration	Dermal
Duration of test	4 weeks
Doses/concentration levels	0.0, 0.5, 1.0, 1.5 g/kg in paraffin oil vehicle
Sex	Males and female (10/sex/group), 72 days old at study initiation
Exposure period	6 hours/day
Frequency of treatment	once/day, 5 days/week
Control group and treatment	Paraffin oil, 2.18 ml/kg/day for 5 days/week
Post exposure observation period	None
Statistical methods	Bartlett's test for homogeneity, Dunnett's test for homogeneous data; modified t-test for non-homogeneous data.
Test Conditions	Animals were housed individually in suspended stainless steel cages with wire mesh bottoms and fronts equipped with an automatic watering system, in a room maintained at 76.1^0F with relative humidity of 56.6% and 12 hour light/dark cycle. Chow diet was provided ad lib. Test article dilutions in paraffin oil (75% v/v) were prepared weekly. Doses of test article were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. Observations for mortality and moribundity were made twice/day, and for clinical signs at least once daily (on dosing days). Dermal responses were scored at initiation and then weekly. Body weight was measured at initiation and then weekly. Food consumption was determined weekly. At sacrifice, gross necropsy was performed, organs/tissues (19/rat) weighed and preserved. Slides prepared for histopathologic examination for the following tissues/organs of control and high dose groups: brain, spinal cord, heart, lungs, thymus, left kidney, right kidney, liver, spleen, sternum, lymph nodes, testes, skin, adrenal glands, urinary bladder, and peripheral nerve.
Results	
NOAEL (NOEL)	NOEL not determined.
LOAEL (LOEL)	LOEL males = 0.5g/kg (increased total WBC count, assigned by reviewer) LOEL females =1.5 g/kg (hematologic alterations, skin irritation, assigned by reviewer)
Remarks	No deaths or moribund rats were observed and no statistically or biologically significant differences in group mean body wt were noted at study termination. No clinical effects were observed that could be attributed to test article administration. Food consumption was significantly decreased in male rats given 1.5g/kg during wks 2 and 3, and in female rats given 1.0g/kg during wk 2. Severe erythema was observed in 1.5g/kg males and females by wk 3 which persisted for the duration of the study. At termination, moderate eschar formation was seen in 10/10 males and 7/10 females. Statistically significant changes in hematology and clinical chemistry parameters after 4 wks of dosing were: dose responsive increase in WBC (57-70%) of males and females in 1.5g/kg group; slight reduction of RBC in males and reduction of HGB and HCT of males and females given 1.5g/kg; elevated platelet counts (10-20%) in 1.5g/kg males and females, reduced total serum protein (10-13%) in 1.5g/kg males and females; reduced serum albumin (9-25%) in high dose males and females; dose responsive reduction in BUN (9-25%) in high dose animals. There were marked increases in segmented neutrophils (200-400%) and lymphocytes (20-30%) in males and females given 1.5g/kg, and an erratic but marked increase in atypical lymphocytes of males in low-high dose groups and increased eosinophils (485%) in 1.5g/kg males. There were several statistically significant but inconsistent changes in organ wt when expressed as

	absolute wt, or per 100 g body wt. but these were not perceived as being biologically significant. There were no histopathological effects noted except those in the skin.
Conclusions	Repeated application of heavy aromatic distillate to male and female rats caused severe skin irritation and significantly decreased food consumption and body wt. Gross and microscopic lesions produced at the site of application, included ulceration, acanthosis and hyperkeratosis. In both male and female rats, treatment with heavy aromatic distillate was associated with significantly elevated WBC counts and mild anemia associated with decreased RBC counts, hematocrit, level of hemoglobin in peripheral blood, and elevated platelet count. The elevated WBC count was related to elevated levels of neutrophils and lymphocytes.
Quality Reliabilities	**2**. Reliable with restrictions. No analysis of test material preparations in paraffin oil.
References	Zellers, J.E. 1985. Four week repeated dose dermal toxicity study in rats using heavy aromatic distillate. Proj. #2063. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other Last changed	Rev. 7/2/2001 (Prepared by a contractor to the Olefins Panel)